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EVALUATION OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE VARIANT (C677T) AS RISK FACTOR FOR BIPOLAR DISORDER

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Abstract

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolism of folate, whose role in bipolar disorder is controversial. The aim of the present study was to assess the risk of MTHFR C677T polymorphism for bipolar disorder. The author performed a meta-analysis and pooled data from individual case-control studies that examined the association between C677T polymorphism and bipolar disorder (meta-analysis: 8 studies, 1457 cases and 2169 controls).The pooled Odd Ratios (OR) were estimated by both fixed effects and random effects models. The meta-analysis with fixed effects showed that there was 71% heterogeneity between the eight studies. The fixed effect pooled OR was 1.07 (95% CI; 0.98 to 1.17) and Cochran Q was 24.13 (df = 7; p=0.0011). The study is significant and shows meager association. The random effect pooled OR was 1.07(95% CI; 0.87 to 1.32) and Cochran Q was 24.13 (df = 7; p=0.0011). The random effect pooled OR was also significant and shows meager association between MTHFR C677T genotype and bipolar disorder.

Key words: Meta-analysis, Bipolar disorder, MTHFR, C677T, Genotype, Polymorphism.

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Abbreviations: **BPD**: Bipolardisorder; **MTHFR**: Methlenetetrahydrofolate; **OR**: Odd Ratio; **SAM**: S-adnosylmethionine; **SAH**: S-adenosylhomocysteine.

INTRODUCTION

Bipolar disorder (BPD), classified as a mood disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM IV). The disorder is characterized by episodes of mania, with elated or irritable-angry mood and symptoms like pressured speech, racing thoughts, grandiose ideas, increased energy, and reckless behavior, alternating with more normal periods and, in most cases, with episodes of depression. These episodes are usually interspersed with periods of relatively normal mood. BPD leads to limited functioning, which often results in decreased productivity in both the personal and the professional areas of the patient's life. The prognosis for patients with BPD is poor, with high rates of relapse, lingering residual symptoms, cognitive impairments, and diminished well being (27). World Health Organization has identified BPD as the sixth leading cause of disability in the world among people aged 15–44 years (48).The prevalence of BPD was thought to be around 1%, but current reported diagnoses indicate that this figure may be closer to 5%. Some studies have suggested that BPD is either under diagnosed (13) or

increasing in frequency, the recent diagnosis of BPD in those under 20 has increased 40-fold from 1994–2003 and has almost doubled in adults in the same period (34).This increased prevalence is mainly accounted for not by an increase in diagnosis of full-blown BPD (which is known as BPD I), but by various softer (i.e., less severe) conditions that fall under the BPD spectrum. Disorders under the BPD spectrum have been grouped based on some overlap in clinical manifestations; however, whether they share the same underlying genetics and pathophysiology is uncertain. Despite advances in its diagnosis and recognition, the underlying neurobiology of BPD remains largely unknown. It is thought that BPD is a multifactorial disease that results from a combination of different genetic profiles, characterized by the presence of various protective and/or preventive genes relative to susceptibility and/or risk genes as well as environmental influences, including chronic stressors and traumatic experiences. The origins of BPD are based partially on genetic factors, for example, studies examining the inheritability of BPD in first-degree relatives show an 8% co-inheritance, increasing to up to 93% in monozygotic twins (10). Several genetic studies suggest that a large number of genes may be necessary for BD inheritance, indicating the complex multifactorial basis for the disorder.

Elevated serum homocysteine levels have been observed in bipolar disorder (29) and a crucial gene (5,10-methylenetetrahydrofolate reductase) involved in folate and homocysteine metabolism has been described as a common risk factor. Methylenetetrahydrofolate reductase (MTHFR) is the essential enzyme in folate mediated one-carbon transfer reactions. It catalyzes the reduction of methylenetetra-hydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and the methyl donor for the remethylation of homocystein to methionine. MTHFR plays a central role in balancing DNA synthesis (which involves 5,10-methylentetrahydrofolate) and DNA methylation (which involves 5-methyltetrahydrofolate). A common variant (677C→T; Ala→Val) of the MTHFR gene is responsible for the thermolabile MTHFR with a specific MTHFR activity less than 50% of the control value in lymphocytes has been identified (15). The homozygous state (677TT) is associated with hyperhomocysteinemia and decreased levels of S- adenosylemethionine (SAM).SAM is the main methyl group donor for all the cellular methylation processes. 677T allele contributes to DNA hypomethylation, which in turn may lead to altered gene expression and this mutation has been reported to be a genetic factor for several diseases like neural tube defects (32,46), Down syndrome (3,11), Cleft lip and palate (33,36) and cardiovascular diseases (4,15). A number of association studies on MTHFR, have focused on possible links between the MTHFR polymorphisms and various psychiatric disorders but no consistent results have been obtained with regard to bipolar disorder. Although some studies (23,26,45) suggest that MTHFR C677T polymorphism is associated with bipolar disorder, some others (2,22,37) do not. So in order to shed some light on these contradictory results a meta-analysis of published case-control studies relating C677T polymorphism and bipolar disorder is carried out. Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small and the statistical power low. Several meta-analysis studies illustrate the utility of the technique in identifying genes of small effects like MTHFR with phenotypes like - schizophrenia(51), diabetes(53) and cancers (50,52,54) etc.

METHOD

Author assessed the association between the MTHFR C677T polymorphism and bipolar disorder by conducting meta-analysis of published case-control genetic association studies.

Inclusion criteria

All research articles that investigate the association of the MTHFR C677T SNP with the risk of bipolar disorder published before May, 2010 were extracted by computer based search of 'Pubmed' database. Only free full case-control genetic association publications were included in the present meta-analysis. The control group included individuals who have no family history of any psychiatric disorder. Genome scans, linkage studies were not included, only studies using validated method for case control studies were included in the present meta-analysis.

Search strategy

Author identified eight eligible studies by searching Pubmed for all publications up to May 2010. Search terms were used "MTHFR","Methylenetetrahydrofolate Reductase", "5,10 methylenetetrahydrofolate reductase", and "C677T" in combination with "Bipolar disorder".

Data extraction

Relevant information's were extracted from all selected studies like- author name, journal name, year of publication and number of cases and controls for each C677T genotypes (CC, CT and TT genotypes). Allelic frequencies for the cases and controls were calculated from corresponding genotypes. Allele frequency was calculated

by simple gene count method according to the following formula:
In a diallelic gene with two alleles, total of frequency of both the alleles should be 1 (p and q are the frequency of two alleles)
p + q = 1
Frequency of p allele = 2 x number of homozygote + number of heterozygote/ 2 x number of samples
Frequency of q allele = 1-p

Population stratification

In any large, randomly mating population, in which there is a constant mutation rate, and no migration or selection against a particular genotype, the proportions of the various genotypes will remain unchanged from one generation to another. To test for population stratification, the distribution of genotypes in control subjects of each individual population was tested for departure from Hardy-Weinberg equilibrium.

Meta-analysis

Author tested heterogeneity between studies using Cochran's chi-square-based Q-statistic and estimated the degree of heterogeneity with I^2 (I^2 = ((Q-(k-1))/Q) ×100%, where k indicates number of studies). I^2 ranges from 0% to 100%. It indicates the proportion of between-study variability in point estimates that was due to heterogeneity rather than sampling error (19,20). An overall OR and 95% confidence interval (CI) was estimated under the Mantel-Haenszel's fixed-effects model (30) if there was no evidence for heterogeneity (I^2 < 50%), otherwise (I^2 = 50%) under the DerSimonian-Laird random-effects model (12). A random effects modeling assume a genuine diversity in the results of various studies, and it incorporates between-study variance into the calculations. The statistical analyses were performed using the program Meta-analysis with Meta-disc (version 1.4).

RESULTS AND DISCUSSION

Total eight articles were found suitable for the inclusion in the present meta-analysis (2,9,22,23,26,35,37,45). Data from eight articles that investigated the association between C677T polymorphism and bipolar disorder were included in the present meta-analysis. The full articles of the retrieved studies were read to assess their appropriateness for meta-analysis. In all the studies, validated genotyping methods were used for the determination of the C677T polymorphism i.e PCR-RFLP. The studies were published between 1997 and 2009. The eight case-control studies provided data on MTHFR C677T genotype for a total of 1457 cases (individuals with bipolar disorder) and 2169 controls. These eight studies were performed in seven different countries like- China (9), Norway (22) , Turkey (35) , Poland (23) , Germany (37) , Japan (2,26) , and Singapore (45) (Table 1).
In all eight studies, total number of cases was 1457, out of which CC genotype was in 651 individuals, CT genotype was in 617 individuals and TT genotype was in 189 individuals. In total 2169 controls, CC genotype was found in 963 individual, CT genotype was found in 965 individuals and TT genotype was found in 241 individuals. In controls, genotypes percentage of CC, CT and TT were 44.39%, 44.49% and 11.11% respectively. In total cases, genotype percentage of CC, CT, and TT was 44.68%, 42.35% and 12.95% respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 2). Allelic frequencies of C and T alleles were also calculated and presented in table 3. In cases and controls, the allele C was the most common.

Table 1. Details of eight studies included in the meta-analysis

S.No.	Author	Population	Controls	Cases	Year	Journal
1.	Chen et al.	China	461	501	2009	Neuroscience Letters,449:48-51.
2.	Jonsson et al.	Norway	177	117	2008	Am. J. Med Genet.,147: 976-982.
3.	Ozbek et al	Turkey	238	197	2008	Prog.Neuropsychopharmaco. Boil. Psychiatry, 32:1331-1337.
4.	Kempisty et al.	Poland	300	201	2006	Neurosci. Lett., 400: 267-71.
5.	Ref et al.	Germany	176	92	2005	Prog.Neuropsychopharmaco. Boil. Psychiatry,29:1162-1168.
6.	Tan et al.	Singapore	121	167	2004	Psychiatr. Genet., 14: 227-31.
7.	Kunugi et al.	Japan	58	143	1998	Mol. Psychiatry, 3: 435-7.
8.	Arinami et al.	Japan	419	40	1997	Am. J. Med. Genet., 74: 526-8.

The pooled Odd Ratios were estimated by both fixed effects (30) and random effects (12) models. The meta-analysis with fixed effects showed that there was 71% heterogeneity between the eight studies (2,9,22,23,26, 35,37,45). The fixed effect pooled OR was 1.07 (95% CI; 98 to 1.17) and Cochran Q was 24.13 (df = 7; p=0.0011). The study was significant and showed meager association. The random effect pooled OR was 1.07 (95% CI; 0.87 to 1.32) and Cochran Q was 24.13 (df = 7; p=0.0011). The random effect pooled OR was also significant and showed meager association between MTHFR C677T genotype and bipolar disorder (Fig. 1). There was a wide variation in the MTHFR 677T allele frequency in the control groups across different populations, ranging from 0.168 in Poland population (23), to 0.382 in an Japanese population (26) (Table 3). In case samples, T allele frequency ranges 0.227 to 0.454, which is slightly higher in comparison to control.

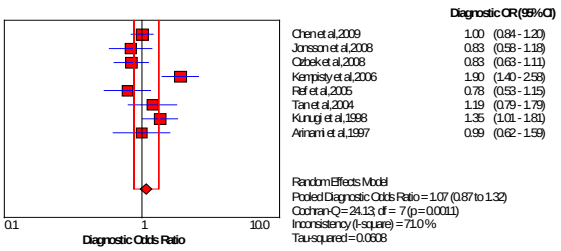

Figure 1. Forest plot: Random effects (RE) odds ratio (OR) estimates with the corresponding 95% confidence interval (CI) for the allele contrast of MTHFR 677 T versus C

The MTHFR gene has a critical role in (i) determining folate and homocysteine levels and (ii) regulation of availability of methyl groups, which are required for epigenetic control of gene expression (47). Folate supplies the substrate for the MTHFR reaction, and in the presence of reduced serum folate and dysfunctional MTHFR showed profound deficits in cellular and physiological processes dependent on MTHFR activity. MTHFR acts in the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetra-hydrofolate (the predominant circulating folate form). The 5-methyltetrahydrofolate is the methyl donor for remethylation of homocysteine (Hcy) to methionine by the vitamin B12-dependent enzyme methionine synthase (MTR). In the methionine cycle, S-adenosylmethionine (SAM), which is the methyl donor for DNA methylation and for other cellular activities, is synthesized through the activity of the methionine adenosyltransferases (MAT) from methionine and adenosine triphosphate (ATP). After transferring the methyl group, SAM is converted to S-adenosylhomocysteine (SAH), a potent inhibitor of cellular methyltransferases. SAH is converted to Hcy catalyzed by SAH hydrolase, being the sole source of Hcy. Thus, the pathologic accumulation of SAH decreases the SAM/SAH ratio and inhibits DNA methylation. Yi et al (49), showed that a moderate elevation in plasma total Hcy level was associated with an increase in plasma SAH concentration and lymphocyte DNA hypomethylation. Decreased intracellular methylation reaction could thus result either from a decrease in formation of SAM or an increase of SAH (41).

The genomic DNA methylation directly correlates with folate status and inversely with plasma homocysteine levels (14). Although there are no conclusive data showing the degree of contributions to hyperhomocysteinemia from environmental studies as well as genetic studies, it is evident that MTHFR deficiency is the most common genetic cause of hyperhomo-cysteinemia. Homocysteine levels are controlled through the transsulfuration or remethylaion pathways (39,41).Regland et al (38) have reported that elevated homocysteine is due to the insufficient remethylation rather than the insufficient trassulfuration in psychotic patients (41).

Several evidences supported that dysfunctional MTHFR variant /folate deficiency might be a risk factor for bipolar or other psychiatric disorders like- (i) Kruman et al (24) reported that in adult mice folate deficiency could significantly reduce the number of proliferating cells in the hippocampus, (ii) Rosenquist and Finnell (40) experimentally proved that in pregnant mice, folate deficiency disrupted progenitor cell replication in the brains ventricular zones, from which most cortical neurons were migrated, (iii)MTHFR –deficient mice generated with small brains with cerebellar abnormalities have 10-fold higher levels of plasma homocysteine, decreased S-adenosyl-methionine (SAM)levels and increased SAH levels (7), (iv) Chen et al (8) reported that MTHFR-deficient mice revealed several genes with altered gene expression, (v) folate restriction in fetal rats was associated with methylation changes and behavioral disturbances during adult life that could then be transmitted to the offspring (25), (vi) Studies on astrocytes and neural stem cells deprived of folate, inhibited their proliferation in culture (31), (vii) hyperhomocysteinemia was consistently reported

Table2. Distribution of the C677T methylenetetrahydrofolate reductase (MTHFR) genotypes in bipolar disorder cases and their corresponding controls are shown

S.N.	Studies	Genotypes						Allele numbers			
		CC		CT		TT		C		T	
		Cases	Control	Cases	Control	Cases	Control	Cases	Control	Cases	Control
1.	Chen et al,2009	178	153	231	235	92	73	587	541	415	381
2.	Jonsson et al,2008	58	80	49	75	10	22	165	235	69	119
3.	Ozbek et al,2008	104	116	76	119	17	22	284	351	110	163
4.	Kempisty et al,2006	108	210	73	79	19	11	289	499	111	101
5.	Ref et al,2005	48	75	34	80	10	21	130	230	54	122
6.	Tan et al,2004	99	80	60	34	8	7	258	194	76	48
7.	Kunugi et al,1998	41	95	74	129	28	34	156	319	130	197
8.	Arinami et al,1997	15	154	20	214	5	51	50	522	30	316
	TOTAL	651 (44.68%)	963 (44.39%)	617 (42.35%)	965 (44.49%)	189 (12.97%)	241 (11.11%)	1919	2891	995	1447

Total No of Samples= 3626
Total No of Control samples= 2169
Total No of Case samples= 1457

Total No of alleles = 7252
Total No of alleles in controls= 4338
Total No of alleles in cases= 2914

*** CC is the wild homozygous individuals, CT is heterozygous and TT is mutant homozygous.**

Table 3. Allelic frequencies of C and T alleles of methylenetetrahydrofolate reductase (MTHFR) genes in bipolar disorder cases and their corresponding controls are shown

S.No.	Studies	Controls				Cases			
		C		T		C		T	
		No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
1.	Chen et al, 2009	541	0.587	381	0.41	587	0.586	415	0.414
2.	Jonsson et al,2008	235	0.664	119	0.336	165	0.705	69	0.295
3.	Ozbek et al, 2008	351	0.683	163	0.317	284	0.721	110	0.279
4.	Kempisty et al, 2006	499	0.832	101	0.168	289	0.722	111	0.277
5.	Ref et al, 2005	230	0.707	122	0.375	130	0.706	54	0.293
6.	Tan et al,2004	194	0.802	48	0.198	258	0.772	76	0.227
7.	Kunugi et al,1998	319	0.618	197	0.382	156	0.545	130	0.454
8.	Arinami et al,1997	522	0.623	316	0.377	50	0.625	30	0.375

in psychiatric patients, (viii) folate deficiency was reported in bipolar disorder patients, (ix) recovery from clinical symptoms was observed in bipolar patients after intake of folate (16) (x) reduced methylation in psychiatric patients compared to healthy controls has been reported in several studies (42), (xi) decreased SAM concentrations were reported in psychotic patients (44) and (xii) psychiatric disorder gene database included C677T variant in the list of the 24 variants with a nominal significant effect on psychiatric disorders (1,43).

According to hyperhomocysteinemia/ hypomethylation hypothesis of bipolar disorder, an aberrant homocysteine-folate metabolism due to reduced MTHFR activity could be present in mother or unborn child and therefore act prenatally (5). Folate deficiency could affect brain in adults or neurodevelopment in fetus through different mechanisms like-(i) folate is an essential precursor for DNA synthesis, its depletion impair neural progenitor division and cortical neurons migration in fetus. (ii)A high level of homocysteine is toxic to dopaminergic neurons (28) and dysfunction of dopaminergic neurons has been reported in bipolar disorder (17). (iii) SAM is methyl donor for COMT, which is involved in the catabolism of serotonin and catecholine and due to decreased concentrations of SAM, catabolism of serotonin and catecholines might be impaired. (iv) Folate deficiency affects neurogenesis in adult brain also. Impaired neurogenesis in the cortical and hippocampal regions of adult brain might lead to decreased brain volume, which is consistently seen in psychiatric patients (25). (v) maternal hyperhomocysteinemia during the third trimester of pregnancy impaired placental profusion, which indirectly leads to reduced oxygen transport to the fetus and fetal hypoxia is a known risk factor for neurodevlopmental disorder.(vi) Folate, vitamin B12, homocysteine and MTHFR are involved in one carbon transfer reaction necessary for the production of monoamine neurotransmitters. (vii) Impaired methylation resulted in abnormal gene expression. DNA methylation is a critical epigenetic modification of the genome that controls many biologic processes, including embryonic development, imprinting and gene expression. Incorrect methylation patterns can affect embryogenesis leading to abnormal development and abnormal gene expression. Although these patterns are established during early life, they are not fixed, and gradual hypomethylation of the genome occurs in most tissues with age, together with aberrant hypermethylation of gene promoter regions. Thus, the correct development of DNA methylation patterns is important not only for early life but also for long-term health benefits, including neurological disease susceptibility (16).

The present meta-analysis has included data for the C677T MTHFR polymorphism from over 1457 subjects who were suffering with bipolar disorder, along with 2169 number of controls. Author found meager association of this polymorphism with bipolar disorder, but there was large and statistically significant heterogeneity in the results of different studies. Higher heterogeneity necessitated a number of subgroup and bias analyses that would yield some interesting findings. The sample size was rather small and further studies, including meta-analysis with large number of subjects is needed for the thorough understanding of the contribution and significance of MTHFR C677T polymorphism in bipolar disorder.

CONCLUSION

In conclusion, pooled analysis of data from eight separate populations including individuals with different ethnicities indicated that the MTHFR 677TT genotype is meagerly associated with bipolar disorder. Low MTHFR enzyme activity may lead severe consequences particularly (i) increase homocysteine level in the blood, (ii) altere/lower methylation status due to low concentration of S-adenosylnethionine (SAM). Folate deficiency could be associated with bipolar disorder or other psychiatric disorder acting either through hyperhomocysteinemia and/or through homocysteine-dependent effect on neuronal progenitor division. In short, MTHFR C677T polymorphism impaired folate and homocysteine metabolism, which resulted in impaired neurogenesis, abnormal methylation and gene expression, dysfunction of dopaminergic neurons due to higher levels of homocysteine in fetus as well as in adult brain (6,14,21) The present meta-analysis showed a large heterogeneity between studies which may be due to genuine differences in the studied population. Future large-scale, population-based association studies are now required to investigate potential gene–gene and gene–environment interactions involving the MTHFR C677T polymorphism in determining bipolar disorder risk.Further studies on function of the MTHFR gene variations, homocysteine levels, folic acid and vitamin B12 in the dynamic

balance of the nervous system and their association with psychiatric disorder may lead to discovery of novel methods for the prevention and treatment of psychiatric disorders.

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